Photo-crosslinking of functionalised lactide oligomers for the fabrication of osteochondral tissue engineering scaffolds

PHOTO-CROSSLINKING OF FUNCTIONALISED LACTIDE OLIGOMERS FOR THE FABRICATION OF OSTEOCHONDRAL TISSUE ENGINEERING SCAFFOLDS

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Summary
In the fabrication of osteochondral tissue engineering scaffolds, the two distinct tissues impose different requirements on the architecture. Stereo-lithography is a rapid prototyping method that can be utilised to make 3D constructs with high spatial control by radical photo-polymerization. In this study, biodegradable resins are developed that can be applied in stereo-lithography. Photo-crosslinked poly(lactide) networks with varying physical properties were synthesised, and by photo-polymerizing in the presence of leachable particles porous scaffolds could be prepared as well.

Introduction
The osteochondral defect is a classical example of a multiple-tissue problem[1]. Tissue engineering of either bone or cartilage imposes different demands on a scaffold regarding its porosity, pore size and pore interconnectivity. Also, local release of tissue-specific growth factors requires a tailored architecture. For the fabrication of an osteochondral tissue engineering scaffold with region-specific architecture, an advanced technique is needed.
Stereo-lithography (SL) is a rapid prototyping technique that allows for the creation of such 3D objects with a well-defined architecture. Its working principle is based on irradiation of a photo-polymerisable resin, causing a liquid-solid transition at a specific location. By irradiating the resin with a computer-driven light source, a solid 3D object can be constructed in a layer-by-layer manner.
To prepare a biodegradable polymer that is applicable in stereo-lithography, we functionalized star-shaped low-molecular weight polymers with double bonds to enable photo-initiated radical crosslinking. In the synthesis of such functionalised oligomers (or macromers), the chemical composition and structural parameters such as the number of arms and the length of these arm can be varied to tailor the physical and biological properties of the resulting network. In this study, methacrylate end-capped lactide macromers of varying arm lengths were synthesised and photo-crosslinked. Films were prepared to evaluate the properties of the networks. Porous scaffolds were prepared by photo-crosslinking in the presence of leachable salt particles. The prepared macromers will be the basis for a resin that can be applied to fabricate osteochondral scaffolds by stereo-lithography.

Experimental methods
Three-armed, methacrylate end-capped PDLLA macromers were synthesised by D,L-lactide ring-opening polymerisation and subsequent functionalization as described by Grijpma *et al*[2]. The arm length was varied by adjusting the monomer to glycerol initiator ratio. Oligomers and macromers were characterised by NMR and DSC. Films containing the macromer and the biocompatible[3] Irgacure 2959 photo-initiator were cast from solution, dried overnight and crosslinked with UV-light in a nitrogen atmosphere at room temperature. The crosslinked films were characterised with respect to their thermal properties (DSC) and mechanical properties (tensile testing). Porous scaffolds were obtained by irradiating a mixture of macromer (30% v/v), NaCl salt particles (70% v/v, sieved to 250-425 μm) and camphorquinone as photo-initiator (5% mol/mol end-groups) with a Kerr Demetron dental curing lamp (intensity 1 W/cm², 470 nm) and subsequent salt leaching in demineralised water. The scaffolds were dried and characterised with scanning electron microscopy.

Results and discussion
In the oligomer synthesis, monomer conversion was typically 99±1% and molecular weights close to the intended values were obtained. 90 up to 96% of the hydroxyl end-groups were

reacted to yield methacrylate end-groups. Figure 1 shows that with an increase in the arm length of the oligomers, Tg increases. A same trend id observed for the macromers. For the resulting networks Tg is independent of the chain length between crosslinks, and in all cases close to room temperature (approximately 20 ^{o}C). In the solvent-cast macromer film, Tg is depressed due to residual solvent, the macromer with an average arm length of 5000 g/mol has a Tg of -13 ^{o}C, and as crosslinking proceeds, Tg increases until the crosslinking temperature is reached. At this point, the system vitrifies and further reactions are quenched. In this way, also the mechanical properties of the network are influenced[4].
Future work will be aimed at crosslinking at higher temperatures and at minimizing the amount of unreacted double bonds to optimize the lactide network properties.

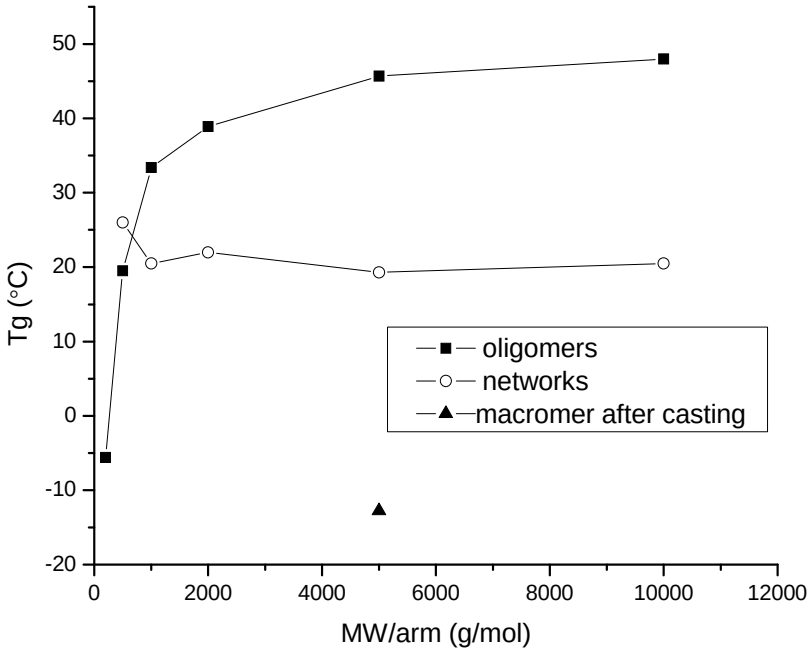

Figure 1: Tg of PDLLA oligomers and photo-crosslinked PDLLA networks

Figure 2 shows a strong dependence of the mechanical properties of the final networks at room temperature on the macromer arm length. With increasing arm lengths the E-modulus decreases and the elongation at break increases. This illustrates the possibility of tuning the properties of the final network by adjusting the macromer architecture.
When the PDLLA macromers are photo-crosslinked in the presence of salt particles, which are subsequently leached with water, porous scaffolds are obtained. The sizes of the resulting pores correspond to the size of the salt particles used (250-425 μm). Figure 3 shows a cross-section of a porous PDLLA scaffold prepared in this manner. Some skin formation of the porous structures can be seen in Figure 4, but nevertheless the largest part of the surface (> 80%) is open and accessible to cells.

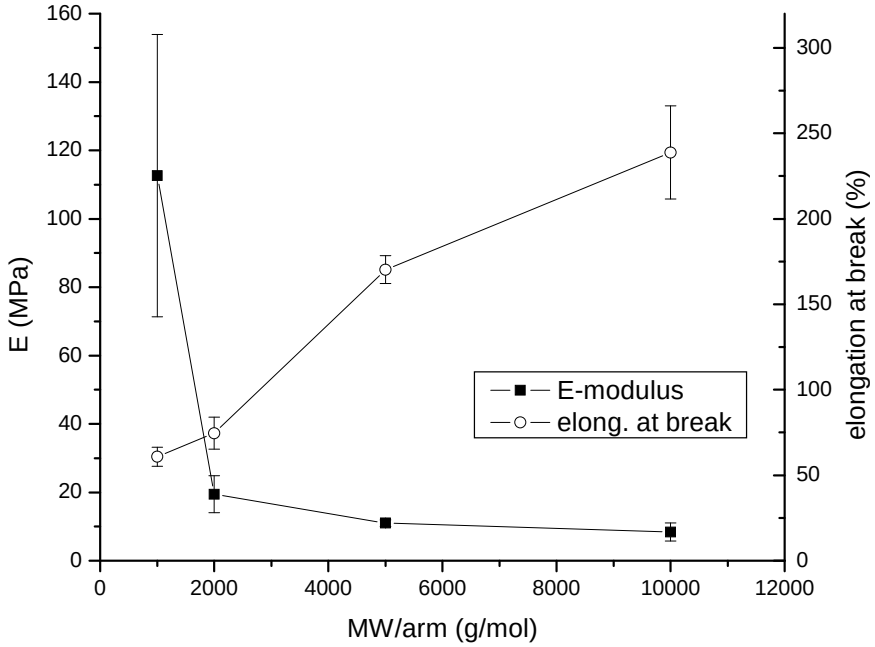

Figure 2: Tensile properties of photo-crosslinked PDLLA networks.

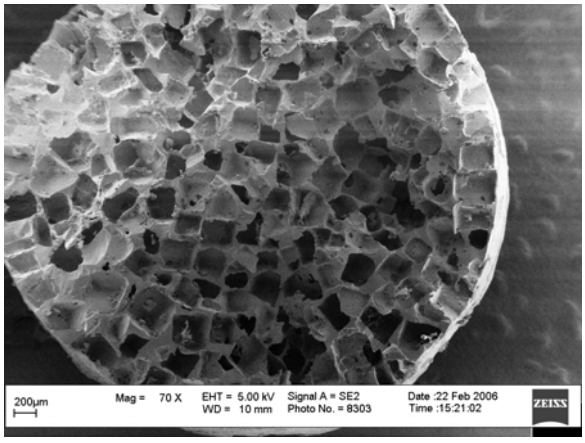

Figure 3: SEM micrograph of porous PDLLA scaffold; cross section. Bar is 200 µm.

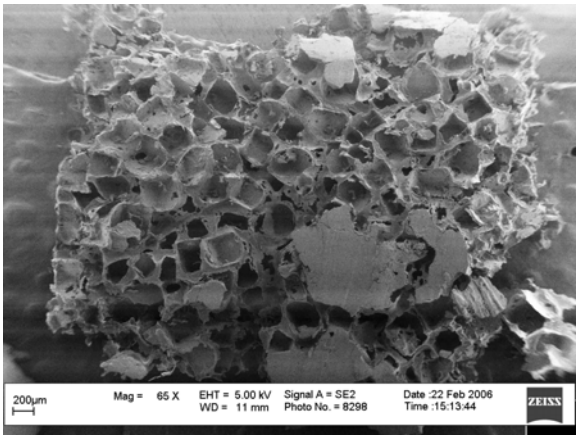

Figure 4: SEM micrograph of porous PDLLA scaffold; outer surface. Bar is 200 µm.

Current work is aimed at utilising the synthesised PDLLA macromers in the fabrication of 3D constructs by stereo-lithography, which will allow us more control over the structural properties of the scaffold. The chemistry involved is similar, but other aspects such as resin viscosity and crosslinking kinetics will become more important. In previous work we prepared 3D constructs with details down to 50 µm on a commercially available stereo-lithography apparatus using a resin based on a liquid poly(trimethylene carbonate) macromer[2]. With this technique we will prepare functional PDLLA scaffolds with well-defined architectures for the tissue engineering of osteochondral defects.

Conclusions

Methacrylate end-capped lactide oligomers (macromers) can be readily synthesised and subsequently photo-crosslinked. The final network properties can be tuned by varying the

macromer arm length. This material can be used as a basis for a stereo-lithography resin to fabricate functional scaffolds with well-defined architectures that are suitable for the tissue engineering of osteochondral defects.

References

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